**PATENT**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| IN RE APPLICATION OF: FUJIMOTO, AKIHIDE ET AL. | EXAMINER: STEVEN C. POHNERT |
| APPLICATION NO.: 10/801,956 | ART UNIT: 1634 |
| FILED: MARCH 15, 2004 | CONF. NO: 2356 |
| FOR: **LOSS OF HETEROZYGOSITY OF THE DNA MARKERS IN THE 12q22-23 REGION** | |

**SECOND DECLARATION OF INVENTOR DAVE S.B. HOON UNDER 37 CFR 1.132**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

1. I am a co-inventor of U.S. Patent Application Serial No. 10/801,956 ('956 Application). I previously submitted a declaration in support of this application dated December 8, 2010.

2. I have reviewed the Office Action dated March 31, 2011 for the '956 Application. This declaration is submitted to support the response to that Office Action.

3. It is my understanding that the examiner questions whether normal individuals not afflicted with cancer have aberrant acellular DNA circulating in their blood. At the time of the invention, it was well known in the art that acellular DNA is present in the blood of healthy and diseased individuals. In

other words, normal individuals not afflicted with cancer have aberrant acellular DNA circulating in the blood. In comparison with patients afflicted with metastatic melanoma, healthy individuals would have normal DNA, i.e., they would not have a loss of heterozygosity (LOH) at the melanoma markers that are the subject of our invention. This comparison was noted in Fujiwara et al., (Cancer Res., 1999, vol. 59, p 1567-1571 ("The study demonstrated that multiple LOH markers can be detected in the plasma of melanoma patients and not in healthy donors.") Fujiwara et al., p. 1567 (last paragraph). We made the same observation (that melanoma patients had LOH, albeit for different microsatellite markers, and healthy donors did not have LOH) in Taback et al., Cancer Res., 2001, vol. 61, 5723-5726, at p. 5724 (see bridging sentence between the two columns), and Nakayama et al., Ann. NY Acad. Sci., 2000, vol. 906, p. 87-98, at p. 91. In other words, our studies showed that the acellular DNA in melanoma patient plasma had LOH at certain microsatellite markers, whereas the acellular DNA in healthy donors does not have LOH at these same microsatellite markers. In Fujimoto et al., Cancer Res., 2004, vol. 64, p.4085-4088; Figure 2 clearly demonstrates, in normal healthy donors' blood, four representative circulating microsatellites DNA of the 12q22-23 region are detected with no LOH. Circulating microsatellite DNA markers are detected in normal and adult humans without LOH. That is one of the reasons why acellular DNA

from a blood sample can be used to predict the probability of survival, prognosis, or cancer therapy efficacy for a metastatic melanoma patient.

4. With respect to control DNA, the '956 Application discloses that a "control DNA sample may be prepared, for example, from a non-neoplastic tissue from the same patient, or from a biological fluid or tissue from a normal person." Typically, that control DNA would not be acellular DNA from a normal patient, but rather DNA extracted from normal cells from the patient or a healthy individual. One of ordinary skill in the art would recognize that control DNA may be derived from any biological fluid or tissue from a normal person, including urine, hair or feces because the DNA from that sample would be diploid in nature and not have the LOH found in the melanoma patient.

5. It is my understanding that the Examiner questions whether Stage I and Stage II melanoma have metastases. They do not. As stated in my prior declaration, in accordance with the official guidelines by the American Joint Committee on Cancer (AJCC), Stage III melanoma is diagnosed by the presence of melanoma tumors that have spread to the regional lymph nodes or have developed in transit metastases or satellites. According to the AJCC guidelines, Stage IV melanoma is diagnosed by melanoma tumors that have spread beyond the regional lymph nodes to distant organ sites in the body. The most common sites of metastases are to the vital organs such as the

brain, lungs, and abdominal organs and soft tissues. In contrast, AJCC Stage I and Stage II melanomas are not associated with metastases. In support of the previous statement, I have attached a copy of the published AJCC guidelines to this declaration as Exhibit 1.

6. It is my understanding that the Examiner questions the applicability of the claimed microsatellite markers to therapies other than the specific biochemotherapy regimen disclosed in Example 2 of the '956 Application. We examined biochemotherapy as a model. Biochemotherapy by definition is made up of immunotherapeutics and chemotherapeutics and the teachings in the '956 application relative to biochemotherapy would be expected to be readily applicable to both immunotherapeutics and chemotherapeutics. One of ordinary skill in the art would understand and accept that that our teachings could be readily applied to any other treatment of metastatic melanoma (such as chemotherapy, radiation therapy, immunotherapy, surgical procedure, and a combinations of those therapies) without undue experimentation because the microsatellite markers are not specific to biochemotherapy.

7. It is my understanding that while the examiner accepts that the Soengas reference (Soengas et al., Nature, 2001, volume 409, pages 207-211) does not teach or direct one of ordinary skill in the art to measure the LOH at microsatellite DNA markers D12S327, D12S1657, D12S393,

D12S1706, and D12S346 from acellular DNA derived from the blood of a metastatic melanoma patient to predict the probability of survival, prognosis, or biochemotherapy efficacy, the examiner questions why one of ordinary skill in the art could not reasonably predict or assume that LOH of microsatellite DNA markers in a melanoma metastatic tumor would be the same in the acellular DNA derived from a metastatic melanoma patient's blood in light of our work in Fujiwara et al. (Cancer Research, 1999, volume 59, pages 1567-1571).

8. The Soengas reference, in part, analyzes Apaf-1 expression and loss of heterozygosity (LOH) at the Apaf-1 locus in metastatic melanoma samples and the Apaf-1 expression in primary melanoma tumors (pp. 207-208), and Apaf-1 expression and Apaf-1 mutations in cell lines derived from metastatic melanomas (p. 208). In assessing LOH at the Apaf-1 locus, Soengas examined the LOH at microsatellite DNA markers D12S1657, D12S393, D12S1706, and D12S346. The LOH at the markers was then associated with the expression or lack of expression of Apaf-1 by measuring mRNA (p. 207 Fig.1 (b)(c)).

9. Notably, Soengas does not teach or direct one of ordinary skill in the art to measure the LOH at microsatellite DNA markers D12S327, D12S1657, D12S393, D12S1706, and D12S346 from acellular DNA derived from the blood of a metastatic melanoma patient to predict the probability of survival,

prognosis, or cancer therapy efficacy. One of ordinary skill in the art could not reasonably predict or assume that LOH of microsatellite DNA markers in a melanoma metastatic tumor would be the same in the acellular DNA derived from a metastatic melanoma patient's blood. For example, in Fujiwara, we demonstrated that while LOH of microsatellite markers can be determined from acellular DNA, the LOH of microsatellite markers from informative metastatic melanoma tumors and informative acellular DNA from metastatic melanoma patients was not identical or predictable.

10. One of ordinary skill in the art would not be enabled, let alone expect to predict the probability of survival, prognosis, or cancer therapy efficacy based upon the LOH data in Soengas because as shown in Fujiwara, merely detecting LOH of a microsatellite marker does not predict prognosis, outcome or efficacy of treatment.

11. In Fujiwara, we demonstrated that while LOH of microsatellite markers can be determined from acellular DNA, the LOH of microsatellite markers from informative metastatic melanoma tumors and informative acellular DNA from metastatic melanoma patient was not identical or predictable. In Fujiwara, we examined the plasma of 76 melanoma patients and 20 healthy patients. Fujiwara, Materials and Methods, Specimens, p. 1568. Of those 76 melanoma patients, 57 had advanced staged melanoma (Stage III and Stage IV) and 19 had early staged melanoma (Stage I and Stage II).

Fujiwara, p. 1567. We analyzed the LOH for a panel of 10 microsatellite markers representing six chromosomal regions in both acellular DNA found in the blood and also DNA found in tumor cells from tumor biopsies. Fujiwara, pp. 1567-1568. Of the 76 patients, we were able to examine matched tumor biopsies and acellular DNA in 40 patients. Fujiwara, Results, p. 1568.

12. Significantly, when we analyzed the 10 markers for their correlation with clinical stage and known melanoma prognosis factors, overall, we found a significant correlation between the number of LOH microsatellite markers within a patient's plasma and the AJCC stage in the 76 patients. Fujiwara, Results, p. 1568. Only one marker (D3S1293), which is not the subject of the present invention, was found to have a significant correlation between LOH detection and clinical progression of disease. Fujiwara, Results, p. 1568. For combinations of markers, three combinations (D9S157 & D3S1293, D9S157 & D1S228, and D11S925 & D3S1293), none of which are the subject of the present invention, were most significant in correlating progression of different clinical stages of disease.

13. However, none of these findings would enable one of ordinary skill in the art to predict the probability of survival, the prognosis, or the biochemotherapeutic efficacy for metastatic melanoma patients based upon the LOH of microsatellite markers in acellular DNA generally, or the markers

of the present invention specifically. That is why Fujiwara states "[t]here was no significant correlation between the frequency of LOH in the plasma or tumor, and standard prognostic factors such as Breslow's thickness or Clark's level." Fujiwara, Results, p. 1569-1570 (bridging sentence between pages).

14. Specific microsatellite markers in acellular DNA can have a profound difference in the prognosis of a metastatic melanoma patient that cannot be predicted based upon the mere cumulative LOH generally, or of the same markers in tumors because the LOH in tumors does not reflect the LOH in acellular DNA for its prognostic value. The present claims are directed to microsatellite markers that have clinical value independent of the cumulative LOH generally, or the LOH in those same markers in tumors.

15. Two years after Fujiwara, we noted in Taback et al., Cancer Res., 2001, vol. 61, 5723-5726 at 5723 that "[t]o date, no major study in solid nonviral-related tumors has determined any significant clinical utility or prognostic value of these free-circulating DNA markers." In Taback, we examined eight microsatellite markers for LOH. Of the eight, only two microsatellite markers (D1S228 and D9S157) had prognostic value (LOH of these makers was found to indicate a poor prognosis for survival. However, as noted in Taback:

> "D9S157 is isolated to chromosome 9p21-22, which is one of the most studied deletion regions in melanoma. It has been shown to occur in up to 60% of cases, and loss of this region has been linked to tumor suppressor gene CDKN2A that is involved in cell cycle arrest and other potential unidentified tumor suppressor gene(s). In one study, evaluating LOH in primary tumors for clinical correlation did not identify a prognostic value for this particular marker." Taback, p. 5726.

16. The finding that LOH at D9S157 in acellular DNA had prognostic value for survival was surprising because it was a well studied microsatellite marker for which LOH in primary tumor did not have prognostic value for survival. Thus, to one of skill in the art, it was unexpected that LOH at D9S157 in acellular DNA would have prognostic value for survival. That finding underscores the unpredictability of the prognostic value of LOH on microsatellite DNA markers in acellular DNA relative to LOH in primary tumor. An increase of LOH is associated with later stage melanoma which is typically associated with poorer prognosis. Simply put, LOH in primary tumor does not predict the prognostic value of LOH on microsatellite DNA markers in acellular DNA.

17. Assessment of acellular DNA offers significant advantages over tumor assessment. Access to tumors is limited because they must be biopsied.

Additionally, as metastatic melanoma often involves vital organs, some tumors maybe inaccessible to biopsy. As melanoma progresses, the ability to monitor patient progression, prognosis, survival and response to therapy is limited because the data points from tumor biopsies is limited. However, the value of circulating DNA in blood is that you can detect multiple times and if related to the tumor progression it can be prognostic and diagnostic. Consequently, the present invention of using acellular DNA microsatellite markers that are predictive for survival, the prognosis, and the efficacy of therapy is not obvious over the prior work of Soengas which is limited to tumor assessment.

*I hereby declare under the penalty of perjury under the laws of the United States of America that all the statements made herein of my own knowledge are true and that all the statements made upon information and belief are believed to be true; and further that these statement are made with the knowledge that willful false statements and the like so made are punishable by fine and imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.*

Dave S.B. Hoon

05-31-11
Date

# Exhibit 1

American Joint Committee on Cancer

# Melanoma of the Skin Staging

7th EDITION

## Definitions

### Primary Tumor (T)

- TX: Primary tumor cannot be assessed (for example, curettaged or severely regressed melanoma)
- T0: No evidence of primary tumor
- Tis: Melanoma in situ
- T1: Melanomas 1.0 mm or less in thickness
- T2: Melanomas 1.01–2.0 mm
- T3: Melanomas 2.01–4.0 mm
- T4: Melanomas more than 4.0 mm

NOTE: a and b subcategories of T are assigned based on ulceration and number of mitoses per $mm^2$, as shown below:

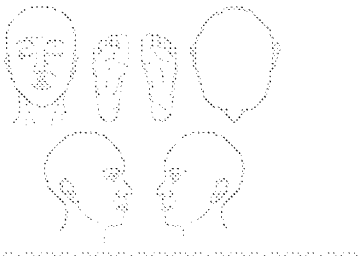
| T CLASSIFICATION | THICKNESS (mm) | ULCERATION STATUS/MITOSES |
|---|---|---|
| T1 | ≤1.0 | a: w/o ulceration and mitosis <1/$mm^2$<br>b: with ulceration or mitoses ≥1/$mm^2$ |
| T2 | 1.01–2.0 | a: w/o ulceration<br>b: with ulceration |
| T3 | 2.01–4.0 | a: w/o ulceration<br>b: with ulceration |
| T4 | >4.0 | a: w/o ulceration<br>b: with ulceration |

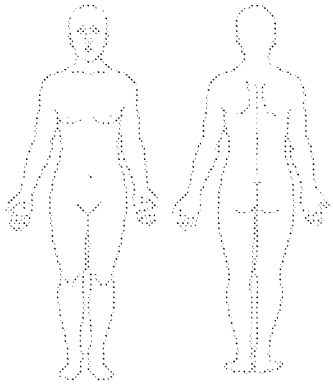
### Regional Lymph Nodes (N)

- NX: Patients in whom the regional nodes cannot be assessed (for example, previously removed for another reason)
- N0: No regional metastases detected
- N1–3: Regional metastases based upon the number of metastatic nodes and presence or absence of intralymphatic metastases (in transit or satellite metastases)

NOTE: N1–3 and a–c subcategories assigned as shown below:

| N CLASSIFICATION | NO. OF METASTATIC NODES | NODAL METASTATIC MASS |
|---|---|---|
| N1 | 1 node | a: micrometastasis[1]<br>b: macrometastasis[2] |
| N2 | 2–3 nodes | a: micrometastasis[1]<br>b: macrometastasis[2]<br>c: in transit met(s)/satellite(s) *without* metastatic nodes |
| N3 | 4 or more metastatic nodes, or matted nodes, or in transit met(s)/satellite(s) with metastatic node(s) | |





### Distant Metastatis (M)

- M0: No detectable evidence of distant metastases
- M1a: Metastases to skin, subcutaneous, or distant lymph nodes
- M1b: Metastases to lung
- M1c: Metastases to all other visceral sites or distant metastases to any site combined with an elevated serum LDH

NOTE: Serum LDH is incorporated into the M category as shown below:

| M CLASSIFICATION | SITE | SERUM LDH |
|---|---|---|
| M1a | Distant skin, subcutaneous, or nodal mets | Normal |
| M1b | Lung metastases | Normal |
| M1c | All other visceral metastases | Normal |
| | Any distant metastasis | Elevated |

| ANATOMIC STAGE/PROGNOSTIC GROUPS | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Clinical Staging[3] | | | Pathologic Staging[4] | | | |
| Stage 0 | Tis | N0 | M0 | 0 | Tis | N0 | M0 |
| Stage IA | T1a | N0 | M0 | IA | T1a | N0 | M0 |
| Stage IB | T1b | N0 | M0 | IB | T1b | N0 | M0 |
| | T2a | N0 | M0 | | T2a | N0 | M0 |
| Stage IIA | T2b | N0 | M0 | IIA | T2b | N0 | M0 |
| | T3a | N0 | M0 | | T3a | N0 | M0 |
| Stage IIB | T3b | N0 | M0 | IIB | T3b | N0 | M0 |
| | T4a | N0 | M0 | | T4a | N0 | M0 |
| Stage IIC | T4b | N0 | M0 | IIC | T4b | N0 | M0 |
| Stage III | Any T | ≥ N1 | M0 | IIIA | T1-4a | N1a | M0 |
| | | | | | T1-4a | N2a | M0 |
| | | | | IIIB | T1-4b | N1a | M0 |
| | | | | | T1-4b | N2a | M0 |
| | | | | | T1-4a | N1b | M0 |
| | | | | | T1-4a | N2b | M0 |
| | | | | | T1-4a | N2c | M0 |
| | | | | IIIC | T1-4b | N1b | M0 |
| | | | | | T1-4b | N2b | M0 |
| | | | | | T1-4b | N2c | M0 |
| | | | | | Any T | N3 | M0 |
| Stage IV | Any T | Any N | M1 | IV | Any T | Any N | M1 |

## Notes

[1] Micrometastases are diagnosed after sentinel lymph node biopsy and completion lymphadenectomy (if performed).

[2] Macrometastases are defined as clinically detectable nodal metastases confirmed by therapeutic lymphadenectomy or when nodal metastasis exhibits gross extracapsular extension.

[3] Clinical staging includes microstaging of the primary melanoma and clinical/radiologic evaluation for metastases. By convention, it should be used after complete excision of the primary melanoma with clinical assessment for regional and distant metastases.

[4] Pathologic staging includes microstaging of the primary melanoma and pathologic information about the regional lymph nodes after partial or complete lymphadenectomy. Pathologic Stage 0 or Stage IA patients are the exception; they do not require pathologic evaluation of their lymph nodes.

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